a mutant cell line Arp 2/3 depleted originated from IA32, evidencing the major role of actin cytoskeleton on the compartmentalization of lipids. Simulations connecting membrane content and actin cytoskeleton density provided insight into the reason for apparent different diffusion modes in different cells.
Whereas lipid hop diffusion could still not be confirmed by SPT experiments using fluorescent tags as markers due to technical limitations, it was now for the first time evidenced by STED-FCS, which is the time-domain counterpart of that technique.

994-Plat
Nanostraw-Mediated Intracellular Delivery: Direct Observation of Cell/Nanotube Interfaces
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As a tool for massively parallel, high throughput molecular delivery, nanostructured platforms have emerged as a delivery technique with unique capabilities. Nanostructured platforms are able to access many different types of cells, even those that resist traditional techniques, and modern fabrication techniques can be used to introduce unique functionalities into individual nanowires or nanotubes on a platform, such as electrical access. Unfortunately, the active delivery process, including the number or percentage of nanostructures that participate, the time scale of delivery, and the mechanisms, whether endocytotic or directly penetrating, are still unknown. Using single event, nanowire-based methods, these important characteristics of delivery are difficult to study and the technique is effectively a black box. We previously reported on a system, the nanostraw platform, capable of time resolved deliveries and in situ observation. Here we introduce a technique using nanostraws to directly observe molecular deliveries to cells. Using a simple, two part delivery assay, we demonstrate not only the direct observation of nanostraw penetration into cells, but also the probability and frequency of cell penetration by nanostraws. We are also able to demonstrate how soon cells accept nanostraw access after plating. Using these nanostraw techniques, we can make the first direct observations of the dynamic high-aspect ratio nanostructure to cell interface during molecular delivery.

995-Plat
Development of New Fluorescent Voltage Sensor Proteins
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Our goal is to develop genetically encoded fluorescent biosensors (FP voltage sensors) and to use them for optical recording of membrane potential in excitable cells. We created a series of FRET (fluorescence resonance energy transfer) based sensors. These constructs contained the voltage sensing domain of CiVSP (Ciona intestinalis voltage sensitive phosphatase) as voltage sensor fused with two fluorescent proteins, UKG (green-emitting fluorescent protein Umi-Kinoko) and mKOK (orange-emitting fluorescent protein Kusabira orange) as donor and acceptor. We used PCR to generate a library of constructs with insertions of the green and orange fluorescent proteins into different regions in CiVSP. Insertion of FRET donor and acceptor at different locations affects plasma membrane expression, FRET signal intensity, and response time constant. We are screening the these FP voltage sensors by examining the FRET signal in response to changes in membrane potential in HEK293 cells. Several novel probes have been identified; some with relatively large signals (>7% $\Delta F/F$) and fast taus (<3 msec). We hope this effort will lead to voltage sensor proteins useful for in vivo recording of membrane potential in neurons.
Supported by US NIH Grants DC005259 and NS054270 and grant WCI 2009-003 from the National Research Foundation of Korea.

996-Plat
Protein Sizing and Conformation Analysis with an Electro-Switchable DNA Chip
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Powerful methods for analyzing proteins and their interactions with small molecules, nucleic acids or other proteins are a cornerstone in the development of new drugs and next-generation disease diagnostics. Commonly, affinity and kinetics of molecular interactions are determined with surface biosensors, which measure the adsorption/desorption of solute target molecules to/from surface immobilized capture molecules. However, information on protein size, shape (folding state), molecular aggregates, or chemically subtle changes such as post-translational modifications, usually remain unrevealed and must be identified in complementary assays.
Here, we show that by analyzing the molecular dynamics of proteins, set in motion by electrically actuated DNA 'levers', it is possible to measure protein size and detect changes in protein structure. Simultaneously, chemical rate constants and dissociation constants are obtained. The method is label-free, uses a parallel microelectrode format for multiplexed assays and microfluidics for low sample consumption. An analytical model based on continuum electrostatic Poisson-Boltzmann theory and the Fokker-Planck equation allows to determine the protein's diameter with sub-nanometer accuracy. Evaluating an extensive set of molecular dynamics data with ligand proteins of differing size, we find excellent agreement between theory and experiment.
The devised concepts open up a novel route for protein analysis on a chip. The relative abundance of antibody fragments in heterogeneous mixtures can be determined, the unfolding of the protein tertiary structure is easily detected and even the ion-induced conformational change of a protein is observed in real-time. Moreover, we demonstrate the revelation of post-translational modifications at the example of a glycosylation and a phosphorylation.
Due to the unprecedented information content about the investigated proteins, offered by this novel technology, we believe that we can provide an essential prerequisite for the progress in early identification of promising leads when screening for biological drug candidates.

Symposium: Membrane Protein Folding

997-Symp
Progress in Membrane Protein Folding
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Integral membrane proteins adopt diverse structures with different stabilites, dynamics and oligomeric states. It is unknown how much of their folding is dictated by the amino acid sequence and how much by the membrane environment. Membrane proteins account for about 25-30% of cellular proteins and their structures are largely hydrophobic and dominated by transmembrane helical bundles. Successful approaches to fold helical membrane proteins have been developed together with advances in kinetic studies *in vitro*. We have combined kinetic, thermodynamic and mutageneis in a study of folding which reveals the reaction free energy and a means to probe the transition state and demonstrates correlations with cellular folding. We also develop in vitro lipid bicelle and bilayer folding systems for membrane proteins. Bicelle properties, as well as the stored curvature elastic stress of model bilayers can be used to optimise the rate, yield and stability of folded protein. We have shown that events such as transmembrane helix insertion, as well as tertiary and quaternary structure formation are altered by the stored curvature stress of the bilayer. We are also progressing our studies to more complex, larger and multi-subunit proteins.
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998-Symp
Do Membrane Proteins Need to Fold in a "Membrane-Mimetic" Environment in Order to Reach a Functional 3D Structure?
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Do membrane proteins need to fold in a ''membrane-mimetic'' environment in order to reach a functional 3D structure?
Since the first demonstration that bacteriorhodopsin can be refolded in vitro from a fully denatured state to a functional one (1), over 20 membrane proteins (MPs) have been folded or refolded in artificial media, and many others have been expressed by cell-free synthesis. The role of the environment in MP folding has however remained somewhat unclear. Which information, which interactions, which constraints does the polypeptide require in order to adopt a functional 3D structure?
Amphipols (APols) are short amphipathic polymers designed to substitute to detergents for handling MPs in aqueous solutions (2). Upon trapping a MP with APols, a water-soluble complex forms, in which the protein is, in general, much more stable than in detergent solution. Among the many applications of